Aptitude for mycorrhizal root colonization in *Prunus* rootstocks

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Abstract.

Eighteen *Prunus* rootstock cultivars were inoculated with three arbuscular mycorrhizal fungi under greenhouse conditions in order to evaluate their affinity for mycorrhizal colonization. The rootstocks were peach-almond hybrids, peaches, plums and cherries of Spanish, French and Italian origin. Mycorrhizal colonization was low in plants inoculated with *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, and *Glomus etunicatum* Becker and Gerdemann. In contrast, *Glomus intraradices* Schenck and Smith, proved to be the most infective endophyte, achieving the highest mycorrhizal colonization rate in most of the rootstocks evaluated. Species of *Prunus insititia* were the only botanical group to show a consistently high affinity for mycorrhizal colonization with *G. intraradices*.

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that increase nutrient uptake by plants, especially phosphorus (P) and several microelements linked to P nutrition (Gerdemann, 1968; Jones et al., 1991). The association with AM fungi occurs in virtually all fruit tree species and can be established naturally in the nursery (early infection) or when transplanted to the field. Measurable growth responses as a direct consequence of inoculation with AMF have been reported in peach (*Prunus persica* L. Batsch) (Gilmore, 1971), peach-almond hybrids (*Prunus dulcis* Mill. Webb x *P. persica*) (Estaún et al. 1994) and apple (*Pyrus malus* L.) (Granger et al. 1983). Mycorrhizal plants have also been shown to benefit from the symbiosis under physiological stress such as nutrient deficient soils (Lindermann, 1988), drought conditions (Nelson, 1987), salinity (Pfeiffer and Bloss, 1988) or when plants are attacked by pests and diseases (Hussey and Roncadori, 1982; Azcón and Barea, 1996).

Knowledge of the mycorrhizal dependency of the most commonly used *Prunus* rootstocks, with emphasis on nematode susceptible rootstocks is limited. Information on newly released commercial rootstocks is not available. The affinity for mycorrhizal infection appears to be cultivar specific rather than species specific (Pinochet et al., 1996), thus the importance of conducting selection for this trait. However, despite all the experimental evidence pointing towards the positive effect of the AM symbiosis on the development of fruit rootstocks, this character has yet to be included in breeding programs. Their mycorrhizal condition could be crucial in providing the rootstocks an increased capacity to withstand stress conditions, such as transplant, salinity, drought, as well as increased tolerance to soilborne fungi and nematodes. This management approach has not been sufficiently explored and could represent a more sound alternative to soil chemical treatments which are expensive, hazardous to field operators and contaminating to the environment.

The purpose of this study is to determine the affinity for mycorrhizal colonization in 18 *Prunus* rootstocks. The materials evaluated are commercial rootstocks of Spanish, French, Italian and American origin, recently introduced into Southern Europe, that have been bred specifically for root-knot nematode (*Meloidogyne* spp.) resistance (Fernández et al., 1994; Pinochet et al., 1999), resistance to iron chlorosis (Socías et al., 1995), tolerance to root asphixia to salinity and drought conditions (Moreno et al., 1995; 2001), and ease of propagation (Felipe, 1989; Socías, 1990).

Materials and methods

Plant material: Seeds, hardwood cuttings, and micropropagated materialñ were supplied by public research institutes and private sources in Spain. A description of the tested rootstocks and their origin are given in Table 1. Seeds of the peach rootstock Montclar were treated with a 5% solution of copper oxychloride for 24 hours, rinsed in water, and stratified in perlite trays that were kept in a storage room at 4º C for 60 days. Seed material was then moved to a 25 $\pm$ 5º C greenhouse to induce germination. Hardwood cuttings of the peach-almond hybrid Garnem (*P. dulcis* x *P. persica*); plums Ademir *(P.domestica* L.*)*, Montizo *(P. insititia L.)* and Monpol *(P. insititia)* were treated for 10 seconds with a 50% alcohol solution that contained 2000 ppm of indole butyric acid (Hansen and Hartman, 1967). Cuttings then were planted in small pots (300 mL) containing a steam disinfected 3:1 (v/v) sand and peat mixture (70ºC during 4 hours). Micropropagated peach-almond hybrids GF-677, Mayor, and Felinem; Barrier and Cadaman peaches *(P. persica* x *P. davidiana* Carr. Franch.); plums Adesoto-101 (*P. insititia*), GF 8-1 (*P. munsoniana* Wight and Hedrick x *P. domestica*), Ishtara (*P. cerasifera*), Julior (*P. insititia* x *P. domestica*), Myrocal (*P. cerasifera*),

Myrobalan 29C (*P. cerasifera*) and Torinel (*P. domestica*); and the cherry rootstock CAB-6P (*P. cerasus L.*) were received as plantlets from Agromillora Catalana S.A., Sant Sadurní d' Anoia, Barcelona, Spain. In vitro plantlets were transfered from agar to 50 mL minipots with peat substrate, and climatized in a high humidity chamber for 24 days until transplant.

Mycorrhizal fungi and inoculation procedures: Three AM fungal isolates, *Glomus etunicatum* Becker and Gerdemann, *Glomus intraradices* Schenck and Smith, and *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, were evaluated. *Glomus etunicatum* was originally isolated from clover (*Trifolium repens* L.) in Cabrils, Barcelona; *G. intraradices* was isolated from citrus (*Citrus aurantium* L.) in Tarragona, Spain and deposited in the European Bank of Glomales as BEG 72; and *G. mosseae* was obtained in 1979 from Rothamsted Experimental Station, England (BEG 116). During transplant to 3.5 L containers, plantlets were inoculated with the AM fungal inocula. Inoculum of *G. etunicatum* consisted of 20 g of rhizosphere soil containing spores (± 60 to 100 per g), hyphae and mycorrhizal root fragments from clover plants. Plants from *G. intraradices* treatments were inoculated with 10 g of rhizosphere soil from one year old leek (*Allium porrum* L.) pot cultures containing heavily infected root fragments with many internal spores (± 500 spores per g of soil inoculum). Plants inoculated with *G. mosseae* received 20 g of rhizosphere soil containing spores, hyphae and mycorrhizal root fragments from one year old leek pot cultures containing 4 sporocarps per g of soil inoculum. The inoculum dosage of the three fungi was calculated in excess to obtain an extensive mycorrhizal colonization of the host root.

Greenhouse experiment: Two to three month-old rootstock materials of uniform growth were transplanted to 3.5 L containers filled with a pasteurized sandy loam textured soil (76% sand; 14% silt, 1% clay; pH 7.5; organic matter content below 1%; and a cation exchange

capacity below 10 meq/100 g soil). An experiment lasting 4 months was established with seven replicates per rootstock and each mycorrhizal combination. The experiment was arranged in a completely randomized design and placed in sand beds in order to reduce soil temperature and humidity fluctuations in the greenhouse. During the course of the study, ambient temperature in the greenhouse ranged between 15 and 34.5º C. Plants were watered as needed, and fertilized with a full-strength Hoagland's nutrient solution once a week (Hoagland and Arnon, 1950).

For assessing mycorrhizal colonization, root samples were collected and stained with 0.05 % trypan blue in lactic acid (Phillips and Hayman, 1970; modified by the procedure described by Koske and Gemma, 1989). The percentage of root colonization was determined using the grid line intersect method (Giovanetti and Mosse, 1980). At least 200 intersects per sample were counted.

Data were analyzed by one-way analysis of variance (ANOVA), and the means were compared by Tukey's multiple range test ($P \geq 0.05$ or $P \geq 0.01$). Percentage of AMF root colonization data were arcsin ($\sqrt{x}/100$) transformed prior to analysis.

Results and Discussion

Colonization with *G. mosseae* was low in general, reaching its highest level in Mayor (34,6%), Montizo (26.7%) and CAB 6P (25.48%) (Fig. 1). This last rootstock did not differ from Julior, GF-677 or Garnem. The remaining rootstocks represented by Montclar, GF 8-1, Monpol, Ademir, Torinel, Adesoto 101, Cadaman, Myrobalan 29 C, Myrocal, Barrier, Felinem and Ishtara., showed a significantly lower level of mycorrhizal colonization (less than 5%).

Glomus etunicatum also showed a low general level of root colonization (Fig. 2). Among these, Torinel (26.62%), Garnem (22.51%), GF 8-1 (8.14%), Montizo (16.31%), Adesoto 101 (9.04%), and CAP 6P (10.99%) presented higher levels. The rest showed varying levels of low mycorrhizal infection.

G. intraradices showed an overall higher root colonization (Fig. 3). The rootstocks Montizo (69.61%), Julior (66.58%), GF-677 (64.93%), Montclar (57.17%), Mayor (52.20%). Adesoto-101 (50.62%), Monpol (48.095), CAP 6P (43.505), Myrocal (38.26%) and Cadaman (38.24%), obtained the highest percentages of root infection. However, the first four rootstocks differed from Ademir, Garnem, Torinel, Myrobalan 29C, Barrier, Felinem, and Ishthara. This last rootstock presented the lowest colonization with this isolate.

Mycorrhizal colonization was low in general in plants inoculated with *G. mosseae* and *G.etunicatum,* reaching the highest level in Mayor (34,6%) and Torinel (26.6%) respectively (Table 3). In contrast, *G. intraradices* proved to be the most infective endophyte. This isolate, originally obtained from citrus in Tarragona, Spain, colonized significantly ($P \leq 0.05$) more root length than *G. etunicatum* or *G. mosseae* in 14 out of 18 rootstocks tested (Figs. 4 and 5). Root colonization in plants inoculated with *G. intraradices* ($P \leq 0.01$) varied between 7.6% in Ishtara and 69.6% in Montizo. The high percentage of root colonization obtained by *G. intraradices* was seldom reflected in enhanced growth. Perennial plants tend to show a delayed growth response to AM colonization. Probably, a longer period of growth would have been necessary to evidence a significant AMF response, as reported in previous experimental work for quince (Calvet et al., 1995) and cherry (Pinochet et al., 1995) where a growth enhancement ocurred seven months after inoculation.

Differences between isolates in their ability to colonize the roots appears to be generated

by the intrinsic characteristics of the plant-fungus interaction. Also, external factors linked to the growth substrate used and other nursery management practices such as fertilization, pest and disease control or excessive shading may affect fungal infectivity, in spite that the experimental conditions for the three isolates was uniform and cautiously monitored. In our study, *G. intraradices* clearly shows to be the most aggressive of the three AMF tested.

It is noteworthy that a similar colonization response to AMF inoculation could be expected in rootstocks with a common genetic origin. This appears to be the case for the *P. insititia* group, as the four rootstocks tested within this group, Adesoto-101, Montizo, Monpol and Julior were highly mycorrhizal when inoculated with *G.intraradices*. The agronomical characteristics of this group are highly advantageous for their specific adaptation to calcareous, heavy and saline soils causing root asphixia and iron chlorosis, common in Mediterranean fruit production areas (Moreno et al., 1995) and for their immunity to root-knot nematodes (Fernández et al., 1995).

Rootstocks included in the *P. cerasifera* group presented different behaviours, showing that there is little relation in their aptitude for mycorrhizal colonization between members of this botanical group. A larger size sample of rootstocks represented *by P. cerasifera* and *P. domestica* would be required to draw any conclusions.

The only cherry rootstock evaluated in this work was CAB-6P, a *P. cerasus* selection belonging to the Cerasus subgenus. CAB-6P showed good receptivity to mycorrhizal colonization, both by *G. intraradices* and by *G. mosseae.* Previous results have been published with another cherry rootstock, Santa Lucia 64, a seed selection of *P. mahaleb (*Pinochet et al., 1995), in which early mycorrhizal infection by *G. intraradices* increased growth and conferred tolerance to the rootstock in the presence of the root-lesion nematode

Pratylenchus vulnus Allen and Jensen.

The Amygdalus subgenus was represented by peach-almond hybrids and peaches that responded well to mycorrhizal inoculation with *G. intraradices* although the percentage of mycorrhizal root ranged from 10.7% (Felinem) to 64.9% (GF 677).

The differences between the three mycorrhizal isolates in their ability to colonize the roots of the same host, also underscore the importance of selecting isolates that are capable of establishing in a short period of time a dynamic symbiosis (Figs. 4 and 5). This also suggests that many peaches and plums are strongly mycorrhizal dependent rootstocks that benefit from the association with this fungal symbiont at early stages of development only 4 months after inoculation, even though AMF have low specificity for the host (Nemec, 1987). No correlation was found between the three fungal isolates in their ability to infect the same rootstock. These results encourage further screening for aptitude for mycorrhizal colonization in micropropagated *Prunus* rootstocks with a larger number of mycorrhizal fungal isolates to determine the best plant-fungus combination.

Early mycorrhizal inoculation can be of special interest to nurseries producing both cuttings and micropropaged material that utilize substrate based potting mixtures that are usually free of mycorrhizae (Estaún et al., 1994). The production of mycorrhizal plants with the adequate selected fungal symbionts may represent an advantage when these young rootstocks are transplanted into the field where the symbiosis can help the plants to withstand stressful conditions.

Results obtained in this study are encouraging, but still, should be regarded as preliminary and constitute a first step in establishing a better knowledge between the association of AMF isolates and new *Prunus* cultivars used as rootstocks. However, to what

extent a given association will be effective in promoting growth, and even more important, in conferring field tolerance to biotic and abiotic stress conditions, still remains to be determined. Association with high root colonization need to be studied for longer periods of time under more stressful field conditions.

We conclude that from the production standpoint, these results clearly indicate the importance of identifying mycorrhizae-rootstock associations that are capable of achieving a high level of root colonization. The most interesting example is the result obtained with the *P. insititia* rootstocks inoculated with *G. intraradices.* Their high aptitude for mycorrhizal colonization with this isolate, indicates that the establishment of micropropagated plants or rooted cuttings from these rootstocks, devoid of mycorrhizae, in an agricultural soil with a low number of AM native fungal propagules or with inadequate AM fungi, would probably cause a delay in their future development. In production areas where these rootstocks are used for their good adaptability, most *Prunus* rootstocks perform poorly. Commercial selections of *P. insititia* plums are fairly new and their genetical manipulation (utilization as parental material in rootstock breeding) has been limited as compared with other European, Japanese and American plums. The high level of affinity between *P. insititia* and *G. intraradices* is, in our opinion, the most significant finding in this study.

The aptitude for mycorrhizal colonization could be associated with a better adaptability of the hostplant to adverse growing conditions, a trait that is seldom considered in breeding programs, overlooking an interesting gene-pool source capable of confering a higher tolerance to specific soil and climate conditions.

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LEGENDS FOR FIGURES

Fig. 1. Percentage of root colonization in 18 *Prunus* rootstocks inoculated with *Glomus mosseae* at 120 days after inoculation. Data are means of 7 replications, transformed to arcsin (√x/100) for analysis. Horizontal bars followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.01$).

Fig. 2. Percentage of root colonization in 18 *Prunus* rootstocks inoculated with *Glomus etunicatum* at 120 days after inoculation. Data are means of 7 replications, transformed to arcsin (√x/100) for analysis. Horizontal bars followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.01$).

Fig. 3. Percentage of root colonization in 18 *Prunus* rootstocks inoculated with *Glomus intraradices* at 120 days after inoculation. Data are means of 7 replications, transformed to arcsin (√x/100) for analysis. Horizontal bars followed by the same letter do not differ according to Tukey's multiple range test ($P \leq 0.01$).

Fig. 4. Comparative root colonization between *Glomus mosseae, G. etunucatum*, and *G. intraradices* in 7 peach rootstocks at 120 days after inoculation. Data are means of 7 replications, transformed to arcsin (√x/100) for analysis. Bars with the same letter for each rootstock do not differ according to Tukey's multiple range test ($P \leq 0.05$).

Fig. 5. Comparative root colonization between *Glomus mosseae, G. etunucatum*, and *G.*

intraradices in 11 plum rootstocks at 120 days after inoculation. Data are means of 7 replications, transformed to arcsin ($\sqrt{x}/100$) for analysis. Bars with the same letter for each rootstock do not differ according to Tukey's multiple range test ($P \leq 0.05$).

Table 1. Source of 18 *Prunus* rootstocks evaluated for mycorrhizal colonization by arbuscular mycorrhizal fungi.

Rootstock	Species/ selection	Origin[z]
Peach Group		
Barrier	*P. persica* x *P. davidiana*	CNR Florence, Italy
Cadaman	*P. persica* x *P. davidiana*	INRA, Bordeaux, France
Felinem	*P. dulcis* x *P. persica*	SIA-DGA, Zaragoza, Spain
GF-677	Natural peach-almond hybrid	INRA, Bordeaux, France
Garnem	*P. dulcis* x *P. persica*	SIA-DGA, Zaragoza, Spain
Mayor	*P. dulcis* x *P. persica*	CIDA, Murcia, Spain
Montclar	*P. persica*	INRA, Bordeaux, France
Plum Group		
Ademir	*P. cerasifera*	CSIC, Zaragoza, Spain
Adesoto-101	*P. insititia*	CSIC, Zaragoza, Spain
GF 8-1	*P. munsoniana* x *P. cerasifera*	INRA, Bordeaux, France
Ishtara	*P. cerasifera*	INRA, Bordeaux, France
Julior	*P. insititia* x *P. domestica*	INRA, Bordeaux, France
Monpol	*P. insititia*	SIA-DGA, Zaragoza, Spain
Montizo	*P. insititia*	SIA-DGA, Zaragoza, Spain
Myrobalan 29 C	*P. cerasifera*	Gregory Bros. California, U.S.A.

Myrocal	*P. cerasifera*	INRA, Bordeaux, France
Torinel	*P. domestica*	INRA, Bordeaux, France
Cherry		
CAB-6P	*P. cerasus*	Univ. Bologna, Bologna, Italy

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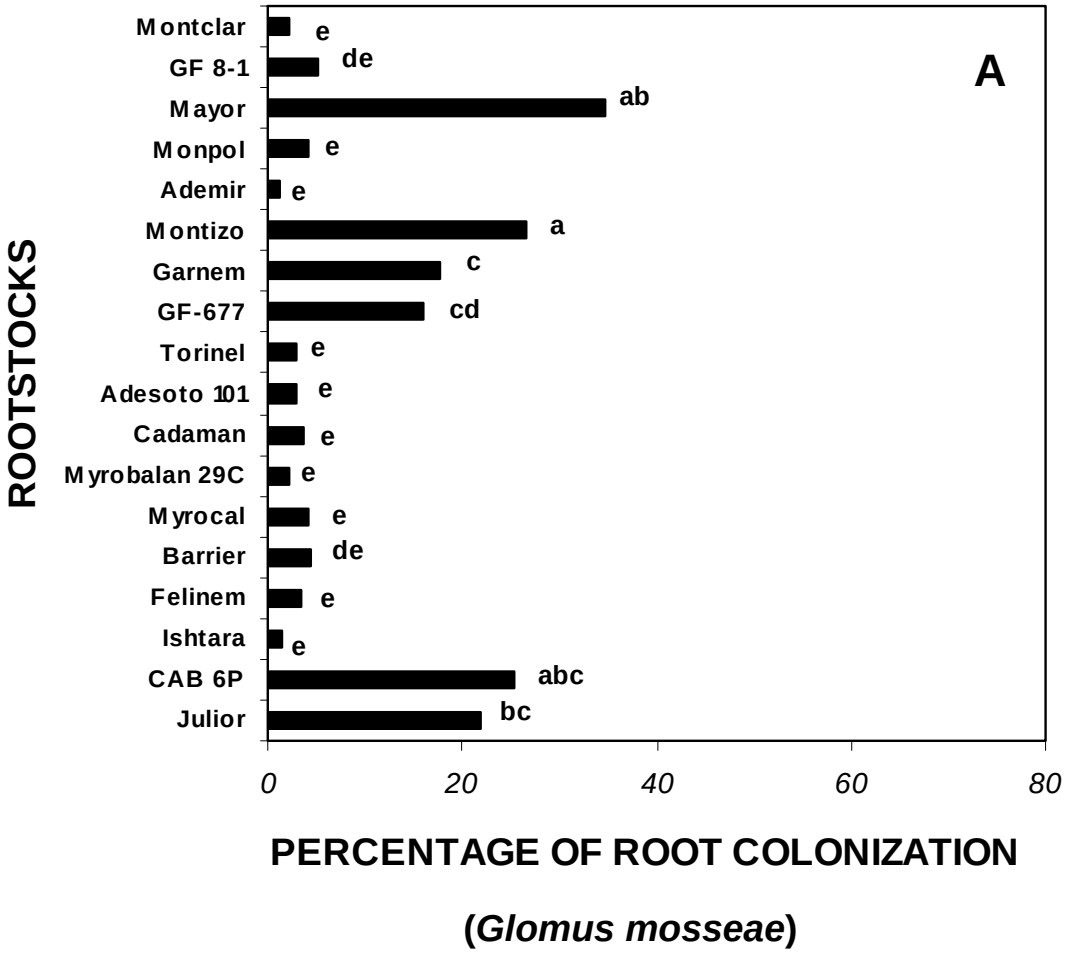
[z] CNR = Consejo Nationale de Recerca; INRA = Institut National de la Recherche Agronomique; SIA-DGA = Servicio de Investigación Agraria de la Diputación General de Aragón; CIDA = Centro de Investigación y Desarrollo Agrario; CSIC = Consejo Superior de Investigaciones Científicas.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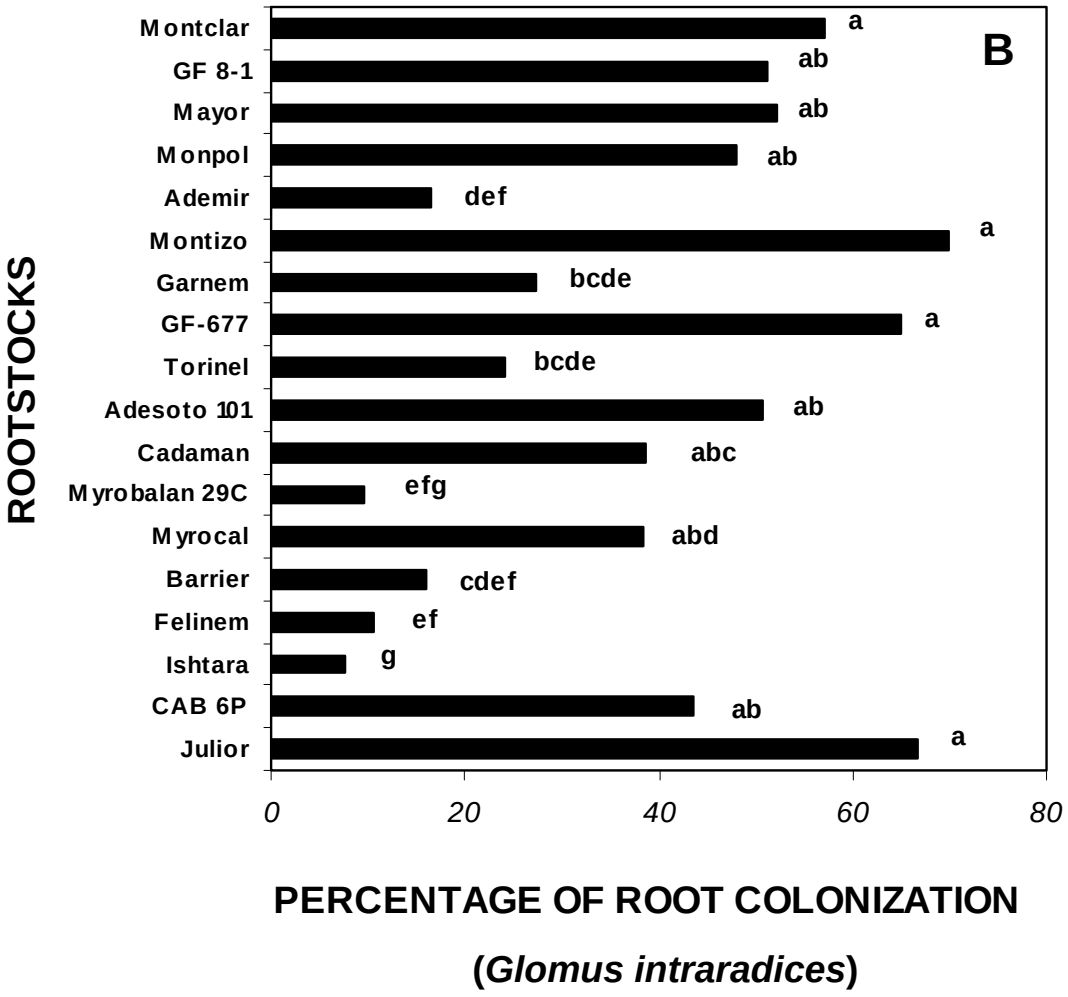
(Glomus mosseae)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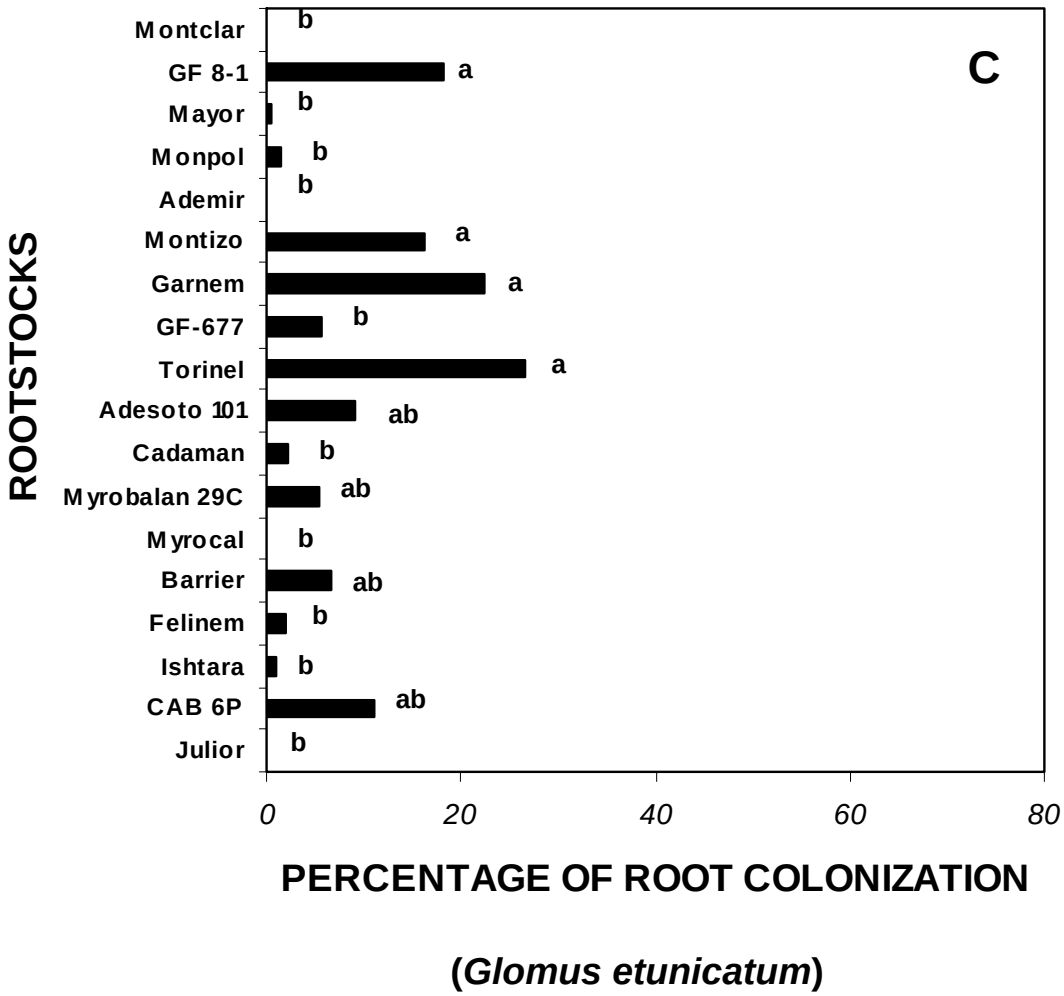
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